

Gain- and loss-of-function approaches in the chick embryo.

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Public Summary:

We describe a method for performing gain- and loss-of-function experiments in early chicken embryos.

Scientific Abstract:

The chicken embryo has been used as a classical embryological model for studying developmental events because of its ready availability, similarity to the human embryos, and amenability to embryological and surgical manipulations. With the arrival of the molecular era, however, avian embryos presented distinct experimental limitations, largely because of the difficulty of performing targeted mutagenesis or transgenic studies. However, in the last decade and a half, a number of new methods for transient transgenesis have been developed that allow efficient alteration of gene function during early embryonic development. These techniques have made it possible to study the effects of gene inactivation or overexpression on downstream transcriptional regulation as well as on embryonic derivatives. This, together with sequencing of the chicken genome, has allowed the chicken embryo to enter the genomic era. While attempts to establish germ line transgenesis are ongoing, methods for rapid, transient spatiotemporally targeted gene alterations have thus again re-established the chick embryo as an important experimental niche by making it possible to apply genetics in concert with classical embryological techniques. This provides a unique tool to explore the role of developmentally important genes (Ishii and Mikawa, 2005; Itasaki et al., 1999; Krull, 2004; Ogura, 2002; Swartz et al., 2001). Transient transfection methods have allowed for efficient mis- and overexpression of transgenes. For long-term analyses, retrovirally mediated gene transfer has particular advantage. For short-term experiments, electroporation and adenoviral-mediated gene transfer methods provide transient expression, largely because of the short persistence time of the transgene within the cell. More recently, Tol2 transposon-mediated constructs have been employed, allowing for integration into the genome and prolonged expression of the transgene (Sato et al., 2007), see Chapter 14 by Takahashi et al., this volume). These methods today are routinely used for gain-of-function analysis, to overexpress or ectopically express genes of interest (Arber et al., 1999; Barembaum and Bronner-Fraser, 2007; Bel-Vialar et al., 2002). Loss-of-function experiments are also possible using electroporation of dominant-negative constructs that act as competitive inhibitors (Bel-Vialar et al., 2002; Renzi et al., 2000; Suzuki-Hirano et al., 2005), morpholino antisense oligos (Basch et al., 2006; Kos et al., 2001; Sheng et al., 2003) that block translation or splicing, or constructs expressing small interfering or small hairpin RNAs (siRNAs or shRNAs) (Chesnutt and Niswander, 2004; Das et al., 2006; Katahira and Nakamura, 2003). Electroporation as the most popular method of the transient transfection into the chick embryos. Electroporation of chicken embryos involves application of an electric field to the exposed tissue that transiently disrupts the stability of the cell plasma membrane, creating reversible pores through which nucleic acids or their analogues can be readily transported into the cytosol. The use of this method for transfection into the vertebrate embryos has been facilitated by adapting the voltage parameters and the type and the duration of the electric pulse. By applying several successive square pulses at a very low voltage, with long rest periods in between, one can successfully deliver a DNA construct or another small charged particle into the cytoplasm, with minimal cell death, high efficiency of the uptake and good embryonic survival rate. The size limit of the DNA molecule that can be transfected in such a way is not yet known, though it is more likely that the size limitation in this procedure (if any) lies within the practical problems of cloning large fragments into the plasmid. We routinely overexpress constructs containing 3-4 kb inserts and coharboring a GFP or RFP reporter whose translation is initiated from an internal ribosomal entry site (IRES), thus allowing easy detection of the electroporated cells.